Salmonellosis in wild birds and its relationship with the infection in finishing pigs

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Abstract
The potential relationship between Salmonella infection in wild birds and pigs was investigated. Feces from pigs, wild birds, and bird droppings or other environmental samples from 25 finishing farms were cultured for Salmonella isolation. In 17 (68%) farms Salmonella was isolated. Out of 57 Salmonella isolates found, 32 (56.1%) were Typhimurium. In 6 (24%) farms the same Salmonella serotype was isolated from samples from different origins and similar AR and PFGE patterns were found, which would support the existence of a transmission cycle of Salmonella infection between birds and pigs in this area. Preventing bird access to farm premises is highly recommended.

Introduction
Pork meat is considered an important source of Salmonella infection for humans in Europe (EFSA, 2008) and the reduction of the prevalence of Salmonella serovars with public health significance in pig herds is considered a major objective in the continent (Regulation (EC) No 2160/2003). The control of pig salmonellosis is difficult. Salmonella spp. are ubiquitous, survive outside the host for months and are able to infect a large variety of wildlife (Murray, 2000). Besides, the Salmonella serovars commonly observed in Europe rarely produce disease, hampering the identification of infected herds. The success of Salmonella control programs relies thus on a good knowledge on the epidemiology of this infection. Factors such as feeding, management, farm design, etc., play a significant role in the spread and maintenance of this infection among pigs (Funk et al, 2004). The role that wildlife may have on the epidemiology of this infection has been suggested many times, but it has been less investigated. With this study we try to gain further insight on the epidemiology of pig salmonellosis in an area of high prevalence in Spain through the search for phenotypic and genotypic similarities among Salmonella isolates from pigs and wild birds that may suggest inter species transmission.

Material and methods
Mist netting was used to humanely trap birds around pig farms. Birds were identified and kept in sterilized dark cages in groups of less than 5 animals of the same species until they defecate. Feces were collected through sterile swabs and birds released after measured and tagged by a licensed bander. Pools of 5 pig feces (from a minimum of 7 pens) and other environmental samples likely contaminated by bird feces were also collected from fattening units. All samples were cultured by triplicate (ISO 6579:2002) and Salmonella isolates serotyped at the National Centre for Animal Salmonellosis (Madrid, Spain) following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).
Salmonella isolates were tested against a panel of 10 antimicrobials (i.e. nalidixic acid, ciprofloxacin, cefotaxime, ampicillin, chloramphenicol, streptomycin, gentamicin, sulphisoxazole, trimethoprim, and tetracycline) using the Kirby-Bauer disk diffusion method (Murray et al, 2003) and following the antimicrobial concentrations recommended by the European Committee on Antimicrobial Susceptibility Testing (Anonymous, 2007) and the Clinical and Laboratory Standards Institute (CLSI) (Anonymous, 2005). Salmonella strains were classified as resistant (R), intermediate (I) or susceptible (S), according to the CLSI guidelines.
Genotyping was carried out by Pulsed-Field Gel Electrophoresis (PFGE) after digestion with XbaI following the CDC standardized laboratory protocol (CDC, 2004). Salmonella Braenderup H9812 was used as molecular size marker (Hunter et al., 2005).
Samples from pigs and wild birds were collected from 25 pig farms. An average of 9.4 (SD=5.6) fecal samples (individual or pools) from wild birds and of 8.3 (SD=3.2) pools of fresh pig feces were collected per farm. In addition, in 23 of these farms environmental samples (i.e. bird feces and samples from aisles, windows or underneath the feed silos) were also collected (an average of 2.7 per farm; SD=1.8).

A total of 234 bird samples, representing 511 wild birds, were collected and 12 (5.1%) were Salmonella positive (Table 1). House sparrows (Passer domesticus) were the most represented species with 44.4% of the samples, followed by European starlings (Sturnus vulgaris) with 6.8%, blackcaps (Sylvia atricapilla) 5.5%, blackbirds (Turdus merula) 3.8%, robyns (Erithacus rubecula) 3.4%, nightingales (Luscinia megarhynchos) 3%, and Sardinian warbler (Sylvia melanocephala) 3%. Other bird species composed less than 30% of the samples. Salmonella was isolated from 4 (33.3%) sparrow samples, 3 (25%) starlings, 1 (8.3%) blackcaps and 1 (8.3%) swallows (Hirundo rustica). Another 3 positive samples belonged to other species.

In 17 (68%) farms Salmonella was recovered from any of the samples taken. In 52% of them it was isolated from pig feces or environmental samples and in 24% from feces from trapped birds. The most common serotype was Typhimurium (56.1%) (Table 1). In 10 (40%) farms Salmonella was recovered from pig feces and bird feces or environmental samples. In 3 farms same Salmonella serotype was isolated from pigs and birds, in 2 from pig and environmental samples, and in one from pig, birds and environmental samples. In 7 out of these 10 (70%) same antimicrobial resistance (AR) pattern was observed between isolates from pig feces and those from bird feces or bird droppings. Multi AR (⊠ 4 antimicrobial families) was found in 25 (59.5%) out of 42 samples analyzed from these 10 farms. The most common pattern was A(C)SSuT (Table 2). PFGE has been performed on Salmonella isolates from some farms. At least in 3 of them identical PFGE profiles were seen in isolates from birds and pigs (Figure 1).

Discussion

The study was carried out in an area of high Salmonella prevalence (Vico et al, 2011). In almost 70% of the farms Salmonella spp. was isolated. It was most frequently recovered from environmental samples indicating its wide distribution within the farms environment (Table 1).

The capture of wild birds around the pig farms was very variable. In some farms less than 5 birds were trapped (20% of the farms) while in other up to 25 bird samples were collected. In addition, the prevalence of Salmonella infection in healthy birds was expected to be low (Tizard, 2004). Despite of these limitations, in 21% of the farms surveyed infected birds were detected. In another 21% of the farms environmental samples, most of them related to bird droppings, were also positive to Salmonella spp. (Table 2). Although some environmental samples might have been contaminated previously by other sources, overall, these findings support the potential risk of Salmonella contamination carried by birds. Samples from house sparrows and European starlings showed the highest prevalence (33% and 25%, respectively). These bird species were more common in the vicinity of pig farms and therefore captured more often. In 68% of the surveyed farms these bird species or their droppings were usually observed inside the fattening units.

In addition to the observation of similar Salmonella serotypes among samples from pigs and birds within the same farms, identical patterns of AR were observed, suggesting the inter species transmission of Salmonella infection in this area. Preliminary results from PFGE showed important similarities among isolates of some of these farms (Figure 1) supporting this conclusion. This information should serve to persuade farmers to keep birds out of farm premises.

Whether birds were victims or responsible of the infection in the pig farm was however debatable. At least in 6 farms bird isolates presented the multi resistance pattern commonly seen in pigs in the region (Vico et al, 2011) and also observed in isolates from these pigs, suggesting a pig to bird transmission. In contrast, in 2 farms susceptibility to all antimicrobials was seen in all isolates. Absence of AR should be more common in birds than in pigs, suggesting the opposite direction of the infection.

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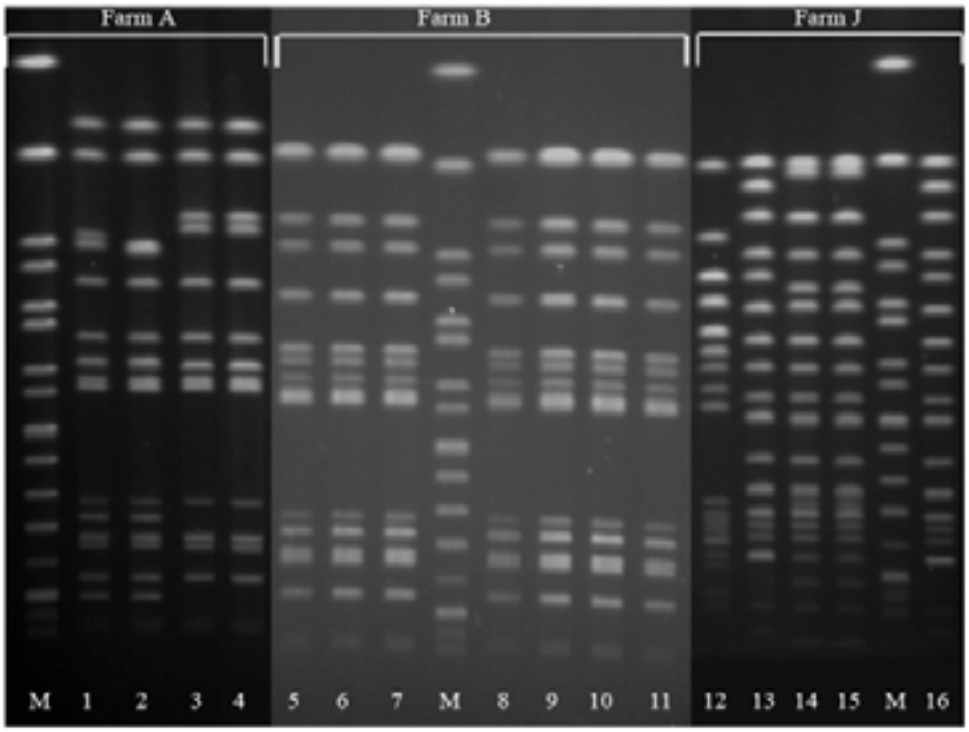
Table 1. Origin and number of samples collected, positive samples after culturing and serotypes identified.

Sample type	Total samples	No. + samples (%)	No. farms	No. + farms (%)	Serotypes (number of isolates)
Pig feces	208	29 (13.9)	25	13 (52)	Typhimurium (19), 1,4,[5],12: i : - (4), Rissen (3), Brandenburgo (2), Anatum (1)
Wild-bird feces	234	12 (5.1)	25	6 (24)	Typhimurium (9), Arizonae (1), Mikawasima (1), Anatum (1)
Environmental	62	16 (25.8)	23	12 (52.2)	Typhimurium (4), Rissen (3), 1,4,[5],12:i:- (3), Bredeney (1), Anatum (2), Reading (1), Houtonae (1), Kapemba (1)

Figure 1. PFGE patterns from Salmonella isolates from three farms



1-11 S. Typhimurium; 12: S. Rissen; 13-16: S. Anatum.
No. 1,2: Pigeon; 3: Blackcap; 4-6, 12 and 13: Pig; 7: European starling;
8: Swallow; 9, 10: House sparrow; 11: bird feces; 14: Feed with bird feces
15: mix of pig and bird feces; 16: House sparrow; M: S. Braenderup H9812

Table 2. List of farms where Salmonella was isolated both from pig feces and other type of samples (bird feces or environmental samples), serotypes identified, and antimicrobial resistance patterns detected.

Farm	Sample type	Origin/description	No. samples	Serotype	AR pattern
A	Pool	Pig	1	Typhimurium	Susceptible
	Individual	Blackcap	1	Typhimurium	Susceptible
	Individual	Rock Pigeon	2	Typhimurium	Susceptible
B	Pool	Pig	2	Typhimurium	Susceptible
	Pool (window)	Bird feces	1	Typhimurium	Susceptible
	Pool	House sparrow	2	Typhimurium	Susceptible
	Pool	Swallow	1	Typhimurium	Susceptible
	Individual	European starling	1	Typhimurium	Susceptible
C	Pool	Pig	1	Typhimurium	ACSSuT-Na
	Individual	European starling	1	Typhimurium	ACSSuT-Na
	Pool (window)	Bird feces	1	4,12:i:-	ASSuT
	Pool	House sparrow	1	Typhimurium	Susceptible
D	Pool	Pig	2	Typhimurium	ASSuT
	Pool (feed silo)	Feed with bird feces	1	Typhimurium	ASSuT-Na*
E	Pool	Pig	1	Rissen	SuT
	Pool (window)	Bird feces	1	Typhimurium	ASSuT
F	Pool	Pig	1	Typhimurium	ASuT-Na
	Pool	Cetti's Warbler	1	Mikawasima	Susceptible
G	Pool	Pig	4	1,4,[5],12: i : -	ASSuT
	Pool (aisle)	Bird feces	1	1,4,[5],12:i:-	ASSuT
H	Pool	Pig	1	Typhimurium	ASuT-Na
	Pool (pen wall)	Bird feces	1	1,4,[5],12:i:-	T
I	Pool	Pig	3	Typhimurium	ASSuT
	Pool	Pig	1	Typhimurium	ACSSuT
	Pool	Pig	2	Typhimurium	ASSuT-Na
	Pool (aisle)	Environment	1	Kapemba	ASSuT-Na
	Pool (window)	Bird feces	1	Rissen	ASSuT
J	Pool	Pig	1	Rissen	ACSuT
	Pool	Pig	1	Anatum	AST
	Pool	House sparrow	1	Anatum	AST
	Pool (aisle)	Pig and bird feces	1	Anatum	AS*SuT
	Pool (feed silo)	Feed with bird feces	1	Anatum	AS*SuT

*Antimicrobial families: A (Aminopenicillins), C (Phenicols), S (Aminoglycosides), Su (Sulfonamides), T (TetracyclineS), Na (Nalidixic acid). * Intermediate resistance.*